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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

Polymorphisms in Genes Involved in Folate Metabolism and Colorectal Neoplasia: A HuGE Review

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Epidemiologic and mechanistic evidence suggests that folate is involved in colorectal neoplasia. Some polymorphic genes involved in folate metabolism—methylenetetrahydrofolate reductase (*MTHFR C677T* and *A1298C*), methionine synthase (*MTR A2756G*), methionine synthase reductase (*MTRR A66G)*, cystathionine β-synthase (*CBS* exon 8, 68-base-pair insertion), and thymidylate synthase (*TS* enhancer region and 3′ untranslated region)—have been investigated in colorectal neoplasia. For *MTHFR C677T* and *A1298C*, the variant allele is associated with reduced enzyme activity in vitro. For the other polymorphisms, functional data are limited and/or inconsistent. Genotype frequencies for all of the polymorphisms show marked ethnic and geographic variation. In most studies, *MTHFR 677TT* (10 studies, >4,000 cases) and *1298CC* (four studies, >1,500 cases) are associated with moderately reduced colorectal cancer risk. In four of five genotype-diet interaction studies, *677TT* subjects who had higher folate levels (or a "high-methyl diet") had the lowest cancer risk. In two studies, *677TT* homozygote subjects with the highest alcohol intake had the highest cancer risk. Findings from six studies of *MTHFR C677T* and adenomatous polyps are inconsistent. There have been only one or two studies of the other polymorphisms; replication is needed. Overall, the roles of folate-pathway genes, folate, and related dietary factors in colorectal neoplasia are complex. Research priorities are suggested.

CBS; colorectal neoplasms; epidemiology; folic acid; *MTHFR*; *MTR*; *MTRR*; *TS*

Abbreviations: CBS, cystathionine β-synthase; CI, confidence interval; MSI, microsatellite instability; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; OR, odds ratio; rpt, repeat; TS, thymidylate synthase.

Editor's note: *This article is also available on the Web site of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).*

Evidence is accumulating for a role of folate in the etiology of colorectal carcinomas and adenomas (1). Many of the genes involved in folate metabolism are polymorphic (2). This paper reviews five polymorphic genes—methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), methionine synthase reductase (*MTRR*), cystathionine β-synthase (*CBS*), and thymidylate synthase (*TS*)—and their associations with colorectal neoplasia.

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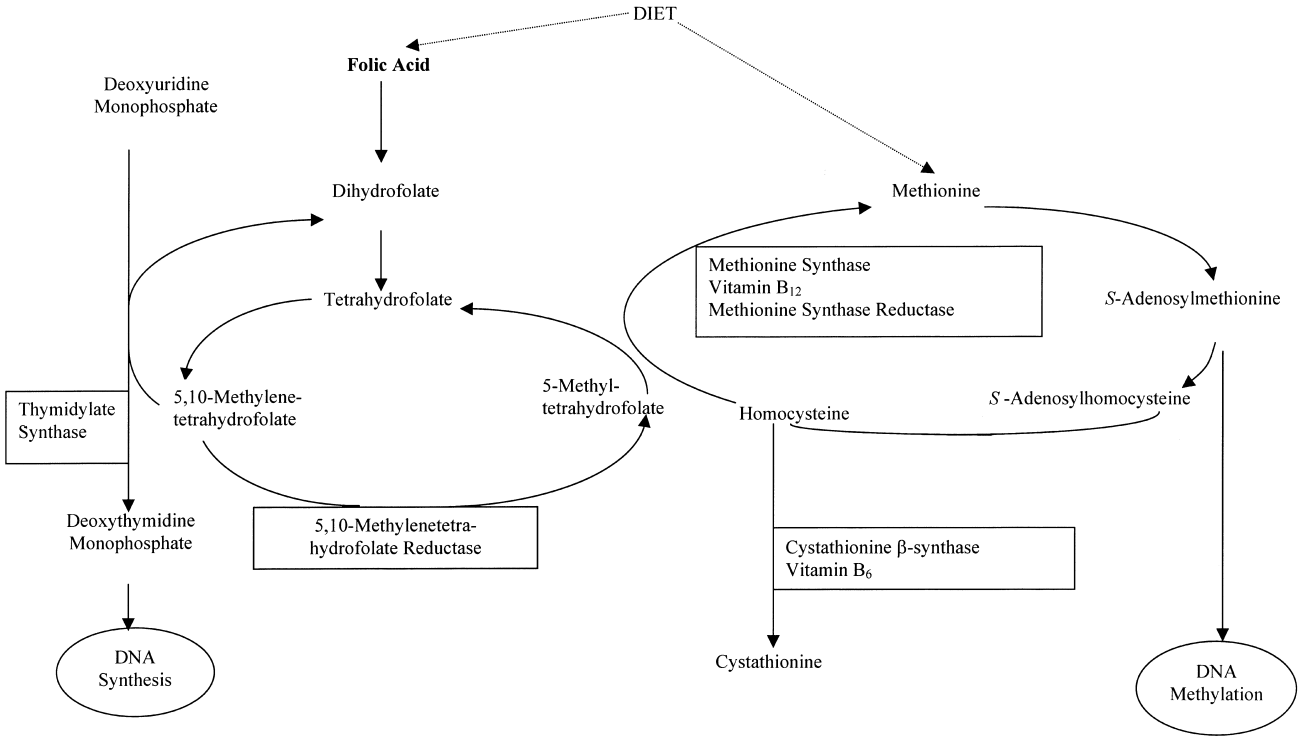

FIGURE 1. The roles of the methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase, cystathionine β-synthase, and thymidylate synthase genes in the metabolism of folate.

GENES

5,10-MTHFR plays a central role in folate metabolism (figure 1), irreversibly converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the primary circulating form of folate. The substrate is vital for DNA synthesis. The product provides methyl groups for synthesis of methionine, a decreased pool of which may affect DNA methylation. The gene encoding 5,10-MTHFR, *MTHFR*, is located at 1p36.3 (3).

MTR, which is essential for maintaining adequate intracellular folate pools, catalyzes the remethylation of homocysteine to methionine, required for production of *S*-adenosylmethionine, the universal methyl group donor. Vitamin B_{12} is a cofactor in this methylation process. The *MTR* gene is on 1q43 (4). MTR is maintained in its active form by MTRR (5), the gene for which, *MTRR*, is located at 5p15.3–p15.2. CBS catalyzes the conversion of homocysteine to cystathionine; vitamin B_6 is required in this reaction. The *CBS* gene is at 21q22.3. TS catalyzes the conversion of deoxyuridine monophosphate to thymidine monophosphate, requiring 5-10-methylenetetrahydrofolate as a methyl donor. The *TS* gene is located at 18p11.32.

Folate status could potentially be perturbed by polymorphisms in these genes. Two mechanisms have been proposed by which folate deficiency could affect malignancy: 1) by causing DNA hypomethylation and proto-oncogene activation and/or 2) by inducing uracil misincorporation during DNA synthesis, leading to catastrophic DNA repair, DNA strand breakage, and chromosome damage (6). Human evidence in support of these mechanisms is limited (6, 7).

GENE VARIANTS

This section describes polymorphisms in the genes and their functional effects. With the exception of *MTHFR*, relatively few studies have investigated relations between the polymorphisms and blood levels of folate and related biomarkers in nondiseased persons. In subjects with medical conditions, it is possible that the condition or its treatment, rather than the underlying genotype, influences biomarker levels. Many studies have been small, with limited statistical power. A potential difficulty in interpretation is that any observed difference in biomarker levels by genotype may not be due to the polymorphism under study but to the presence of another polymorphism. Equally, a failure to observe differences in biomarkers by genotype could be due to the presence of another polymorphism with opposing functional effects. So far, there has been little investigation of the effects of combinations of polymorphisms. With regard to *MTHFR C677T*, only red cell folate measured by microbiologic assay is reliable; results of the radioimmune assay are biased (8). There is differential detection by the assays of various intracellular folates, the distribution of which is related to *MTHFR* genotype (9). Whether red cell folate

results measured by radioimmunoassay are biased for other polymorphisms in the folate-pathway genes is not known.

MTHFR

Several polymorphisms in the *MTHFR* gene have been reported, and two have been investigated in colorectal neoplasia: 1) C→T at nucleotide 677, leading to an alanine to valine conversion in the protein (10); and 2) A→C in exon 7, causing an alanine to glutamate protein change (11, 12). These polymorphisms are located 2.1 kb apart. The other polymorphisms—T1059C, T1317C, and G1793A (12–14)—are not discussed further in this paper.

For *C677T*, compared with homozygotes for the common variant (*CC*), heterozygotes have 65 percent of their enzyme activity levels in vitro and those who are homozygous variant (*TT*), 30 percent (15). From the microbiologic assay, compared with *CC* homozygotes, heterozygotes have 10 percent lower and *TT* homozygotes 18 percent lower red cell folate levels (16). Persons with the *TT* variant also have lowered plasma folate and vitamin B_{12} levels and raised homocysteine levels (17, 18). In two studies, the association with homocysteine held only when folate status was low (19, 20); in another, it occurred only when riboflavin status was poor (21). Regarding *MTHFR* and DNA methylation, one small study found that DNA from subjects with the *TT* variant had a significantly higher methyl group acceptance capacity than DNA from subjects with the *CC* variant (22), but this finding was not confirmed in a larger study (23). In 292 subjects (66 percent of whom had coronary atherosclerosis) selected by *MTHFR* genotype (187 *CC*, 105 *TT*), DNA methylation status was affected by genotype among only those with lower plasma folate levels; subjects with the *TT* variant who had lower plasma folate concentrations had lower methylation levels than all other groups of subjects (24). A few studies have investigated *MTHFR* and uracil misincorporation, DNA strand breaks, or genetic instability in vivo and in vitro, with inconclusive results (23, 25–27).

For *A1298C*, enzyme activity in vitro is decreased in homozygotes variants (*CC*) and, to a lesser extent, in heterozygotes compared with those without the variant (11). Studies of *A1298C* and plasma folate and homocysteine are inconsistent (12, 28–31), which may be due to methodological reasons (e.g., non-population-based study, small sample size), or it may be that there is a relation that depends on the status of folate and/or related nutrients. Enzyme activity in vitro for compound heterozygotes (i.e., heterozygotes for *C677T* and for *A1298C*) is unclear (29).

MTR

The A-G polymorphism at position 2756 in the protein binding region of *MTR* replaces aspartic acid with glycine (32). Most studies suggest that plasma homocysteine level is lower in those with the rarer, *G*, than the more common, *A*, allele (18, 33–36). One study found significantly higher plasma folate levels in *GG* than in *AA* subjects (34), but this finding was not observed in another study (18). Evidence on red cell folate and on plasma vitamin B_{12} and vitamin B_6 is very limited (18, 35, 37).

MTRR

The *A66G* polymorphism in the *MTRR* gene results in the substitution of isoleucine with methionine at codon 22 (5). In two studies, subjects homozygous for the common allele (*AA*) had elevated homocysteine levels compared with those who had other genotypes (38, 39); in a third study, genotype was not a significant predictor of homocysteine level (40). No associations were found between genotype and serum folate, vitamin B_6, or vitamin B_{12} in the single known study (38).

CBS

Many mutations and several polymorphisms in the *CBS* gene have been reported (41). To our knowledge, the only variant investigated in colorectal neoplasia is the 68-base-pair insertion in the exon 8 coding region. Four studies found lower plasma homocysteine levels in persons carrying the insertion than in those without, although the difference was significant in only one (35, 36, 39, 42). One study suggested that the effect was modulated by plasma vitamin B_6 concentration (43); another suggested an interaction with *MTHFR C677T* (35). The one available study that we know of found no associations between genotype and red cell folate or plasma vitamin B_{12} level (35).

TS

The *TS* enhancer region contains a series of 28-base-pair tandem repeats. Two repeats (2 rpt) or three repeats (3 rpt) are most common, with 3 rpt occurring most frequently. More repeats have been observed but are rare (44, 45). In vitro, compared with the double repeat, the triple repeat has been associated with 2.6-fold greater thymidylate synthase expression (46). Among 497 Singapore Chinese, plasma folate levels were significantly lower, and homocysteine levels nonsignificantly higher, in 3 rpt/3 rpt subjects than in those with other genotypes (47). When *MTHFR* and *TS* were considered together, plasma folate levels were highest (15.3 nM) in *677CC* or *677CT* and not 3 rpt/3 rpt subjects, intermediate (13.8 nM) in *677CC* or *677CT* and 3 rpt/3 rpt subjects, and lowest (11.6 nM) in *677TT* subjects (irrespective of *TS* genotype).

The 3′ untranslated region contains a 6-base-pair deletion at base pair 1494, the functional consequences of which are not known (48). The two polymorphisms appear to be in linkage disequilibrium (48).

Refer to the Appendix for Internet sites pertaining to the genes discussed in this review.

POPULATION FREQUENCIES

This section includes information on studies reporting genotype frequencies in persons without cancer or other diseases. Using appropriate Medical Subject Headings (MeSH) and text words, we searched MEDLINE, EMBASE, and PubMed databases for papers published from 1990 to December 2002. Further relevant articles were identified by hand-searching reference lists in published papers. *MTHFR*

frequencies are from the Human Genome Epidemiology (HuGE) reviews by Botto and Yang (49) and by Robien and Ulrich (50). The *A1298C* data reported by Robien and Ulrich are augmented with results from less-studied geographic areas and ethnic groups. For the studies tabulated here, Hardy-Weinberg equilibrium of the genotype frequencies was assessed by using the Pearson χ^2 test.

MTHFR

There is considerable ethnic and geographic variation in the frequency of the *C677T* variant (49). The *TT* prevalence ranged from around 1 percent in Black populations in the United States, sub-Saharan Africa, and South America to more than 20 percent in US Hispanics, Colombians, and Amerindians in Brazil. *TT* genotype frequency in White populations in Europe, North America, and Australia was 8–20 percent. In Europe, there appears to be a trend of increasing frequency of the variant from north to south. Twelve percent of Japanese were *TT* homozygotes.

For *A1298C*, the *CC* prevalence in North American studies, which included mainly White subjects, was 7–12 percent (50). In four Hispanic series ($n < 90$), the frequency was 4–5 percent (51–54). In two African-American series, 2 and 4 percent were *CC* subjects. In Europe, the prevalence of *CC* ranged from 4 to 12 percent in most studies. In two northeast Scotland series of subjects randomly selected from general practitioner registers, the frequencies were 15 percent (95 percent confidence interval (CI): 11.8, 19.2) and 18 percent (95 percent CI: 9.5, 30.4) (55, 56). In Chinese, Japanese, and Hawaiian populations, 1–4 percent were *CC* (50, 54) subjects. In the single studies in Brazil, Morocco, South Africa, and Turkey and among Israeli Jews, the frequencies were 6 percent (95 percent CI: 2.8, 9.6), 3 percent (95 percent CI not available), 4 percent (95 percent CI: 1.4, 9.9), 6 percent (95 percent CI: 1.7, 14.8), and 13 percent (95 percent CI: 9.7, 16.5), respectively (31, 33, 57–59).

In some series, but not all, a few persons with three or four variant alleles (i.e., *677TT/1298AC*, *CT/CC*, *TT/CC*) have been reported (35, 60–64).

MTR

In Japanese, Chinese, and Korean populations, the frequency of the *GG* genotype was 2–3 percent (18, 32–37, 54, 65–82; Web table 1). (This information is described in the first of four supplementary tables; each is referred to as "Web table" in the text and is posted on the Web site of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm) as well as on the *Journal*'s Web site (http://aje.oupjournals.org/).) In most European series, approximately 3 percent of the subjects had the *GG* genotype. Frequencies from all but two North American studies were 1–5 percent. The frequency was 10–11 percent in these two series—one of White children and their mothers in Canada and the other of White persons in Hawaii. In the single African-American population, 6 percent (95 percent CI: 4.3, 8.7) of the subjects had the *GG* genotype. In three studies, the genotype frequencies were not in Hardy-Weinberg equilibrium (73–75).

MTRR

The lowest reported prevalence of *GG* homozygotes was 8–10 percent in Japanese in Hawaii and in Hawaiians (5, 14, 38–40, 54, 83–85; Web table 2). Among 558 subjects in Northern Ireland, 12 percent (95 percent CI: 9.1, 14.6) were *GG* homozygotes, but this series was not in Hardy-Weinberg equilibrium. In most of the remaining series, the frequency was 19–29 percent. Among 97 African Americans and 96 Hispanics, the frequencies were 42 percent (95 percent CI: 32.3, 52.7) and 50 percent (95 percent CI: 39.6, 60.4), respectively.

CBS

Homozygosity for the 68-base-pair insertion is rare in all populations (35, 36, 39, 42, 54, 65, 70, 71, 77, 82, 86–98; Web table 3). The highest reported frequency was 3 percent among Blacks from Brazil and Africa. In four other series, the homozygote prevalence also reached 3 percent, but the genotype frequencies were not in Hardy-Weinberg equilibrium (42, 70, 71, 96). In Europe, Australia, and most US populations, the frequency of heterozygotes was 8–19 percent, with most around 13–15 percent. Two Japanese series found no heterozygotes. Heterozygosity occurred in 5 percent (95 percent CI: 1.6, 11.3) of the single Chinese series.

TS

In three studies in the United Kingdom, and in three of mainly White populations in the United States, 19–23 percent of subjects were 2 rpt/2 rpt (44–47, 99–102; Web table 4). The prevalence was 14–20 percent in two African and one African-American series and 17 percent among volunteers born in four southwest Asian countries living in Scotland. Two to 4 percent of two Chinese populations were homozygous variant. In all studies, genotype frequencies were in Hardy-Weinberg equilibrium.

In a single study of US Whites, 10 percent (95 percent CI: 7.7, 12.5) were homozygotes for the 3′ untranslated region deletion (102).

Combinations of genotypes

Most studies reporting frequencies of combinations of genotypes are small (33, 35, 70, 80, 94, 103). In the largest, of almost 1,300 males in the United Kingdom, 8 percent carried the *CBS* 68-base-pair insertion and the *MTHFR T* allele; 5 percent of subjects had the *CBS* 68-base-pair insertion and the *MTR G* allele; and 20 percent carried both the *MTR G* and *MTHFR T* alleles (35).

Comments on studies of population frequencies

Few of the studies reviewed here were population based; many relied on convenience samples. Selection and partici-

pation biases may therefore explain some of the apparent variations in genotype prevalence. In a few studies, genotype frequencies were not in Hardy-Weinberg equilibrium. Although lack of Hardy-Weinberg equilibrium might indicate that the series were subject to selection or participation biases, there are other reasons why Hardy-Weinberg equilibrium might not hold, including migration or genotyping error (104). Many of the studies are relatively small, so the estimates of genotype frequency lack precision.

In many studies, the ethnic makeup of the participants is not described. Most well characterized are White populations in the United States and western Europe. Other populations, geographic areas, and ethnic groups, particularly in Africa, Asia (other than Japan), and South America, have been less studied. The generalizability from, for example, one "Black African" population to another may be limited since it is not always straightforward to establish ethnicity (105).

DISEASE

An estimated 945,000 new cases of colorectal cancer were diagnosed worldwide in 2000, and 492,000 persons died from the disease (106). Two thirds of incident cases occur in developed countries, where it is the third most common cancer in males and second most common in females (107). There are substantial international variations in incidence (108). Sixty to 70 percent of colorectal cancers arise in the colon (108).

Although most evidence is indirect, the majority of colorectal carcinomas are believed to develop from adenomatous polyps (109). Hyperplastic polyps may be precursors of some right-sided colon cancers (110). Investigation of the first occurrence, and the recurrence, of polyps may reveal factors important in early stages of the neoplastic process.

Fewer than 10 percent of incident colorectal tumors are due to hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis (111). When these syndromes are excluded, there is still familial aggregation of cancers and adenomas (112–114), which is unlikely to be entirely accounted for by familial clustering of environmental factors (115). This information points to the potential importance of genetic susceptibility factors, and the interaction of these with each other and with environmental factors, in the disease causation.

The studies of Japanese migrants to the United States in the 1960s revealed the overwhelming importance of environmental factors in colorectal cancer etiology (116). Established risk factors for the disease are shown in table 1 (109, 117–125).

Although diet appears to be important in colorectal cancer (120), it has been difficult to identify the specific components involved. Observational epidemiologic evidence shows that a high vegetable intake is related to decreased risk (120), although recent work suggests that the relation is complex (124, 125). Vegetables, particularly green, leafy vegetables, are a major source of folate. The majority of prospective and case-control studies of serum folate, red cell folate, or reported dietary or total folate intake are compatible with inverse associations with colon cancer and adenomas (17, 54, 76, 125–146). There is no consistent association between rectal cancer and folate intake (126, 131, 133–135, 137, 138). One small trial of folic acid supplementation in persons from whom polyps had been removed observed a reduced recurrence rate in the supplemented group (147). Some studies are compatible with a positive association between alcohol intake, which adversely affects folate metabolism (148), and colorectal neoplasia (109). A "low-methyl diet," comprising high alcohol intake and low folate and methionine (and/or vitamins B_6 and B_{12}) intakes, has been associated with increased colon cancer risk (126, 130, 132, 140).

Internet sites providing data and information on colorectal neoplasia are contained in the Appendix.

TABLE 1. Environmental factors associated with colorectal cancer

Increasing risk	Reducing risk
Excess weight*	Physical activity†
Tobacco smoking‡	Hormone replacement therapy§
Alcohol¶	Aspirin and other nonsteroidal antiinflammatory drugs#
	Vegetables**

* Bergström et al. (117); International Agency for Research on Cancer (IARC) Working Group (118).
† IARC Working Group (118).
‡ Giovannucci (119).
§ Beral et al. (121); Rossouw et al. (122).
¶ World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR) (120); Cotton et al. (109): results of studies are heterogeneous.
# IARC Working Group (123).
** WCRF/AICR (120); Terry et al. (124); Flood et al. (125): results of studies are heterogeneous.

ASSOCIATIONS

This section appraises studies of the polymorphisms and colorectal neoplasia risk. These studies were identified by using the search strategy described above with the addition of disease-specific Medical Subject Headings and text words.

MTHFR

C677T. To our knowledge, there have been 10 cancer studies: five in the United States, two in the United Kingdom, and one each in Australia, Mexico, and Korea (17, 54, 56, 98, 149–155; table 2). Two included only colon cancers (150, 154); the remainder included colon and rectal tumors. On the basis of the functional effects of the polymorphism, and the inverse association between folate status and disease, it might have been expected that the variant would be associated with increased disease risk. In contrast, seven studies were consistent with reduced risk in homozygous

TABLE 2. Studies of the *MTHFR C677T* genotype and colorectal carcinoma, with relative risks and 95% confidence intervals**

Study area	Cases		Comparison group				Comparison	Relative risk	95% CI*	Adjustment factors	Reference no.
	Type	No.	Type	No.	% *TT*	95% CI					
Australia†	Patients undergoing surgery for colorectal cancer at a hospital in Western Australia during 1985–1998; Duke's stage B or C; 46% male; 48% aged <70 years	501	"Healthy" persons from Western Australia; aged 20–92 years; 81% aged <70 years	1,207	11.0	8.4, 14.0	*TT* vs. *CC*	1.03‡	0.71, 1.49		98
							CT vs. *CC*	0.75‡	0.60, 0.95		
Korea	Patients undergoing an operation for colorectal cancer at two centers; 51% male	200	"Healthy" unrelated adults without colorectal cancer; source not stated.	460	16.1	12.8, 19.8	*TT* vs. *CC*	0.81‡	0.46, 1.42		151
							CT vs. *CC*	0.94‡	0.64, 1.39		
Mexico†	Patients with colorectal cancer	74	"Asymptomatic" subjects; source not stated	110	21.8	14.5, 30.7	*TT* vs. *CC*	1.61‡	0.62, 4.19		152
							CT vs. *CC*	1.83‡	0.84, 4.11		
United Kingdom: Scotland	Residents of Grampian who had a first primary, histologically confirmed, colorectal cancer diagnosed in 1998–2000; 57% male; median age, 70 years	251	Persons randomly selected from lists of all those registered with general practitioners in Grampian; frequency matched to cases on age and sex; 51% male; median age, 62 years	394	11.9	8.9, 15.5	*TT* vs. *CC*	0.93	0.66, 1.32	Age, sex	56, 153
							CT vs. *CC*	0.72	0.41, 1.28		
United Kingdom: Perth, Dundee, Leeds, York	Patients with incident colorectal cancer from four hospitals; aged 45–80 years; Caucasian; no history of familial adenomatous polyposis, inflammatory bowel disease, ulcerative colitis, diverticular disease, or previous malignancy	490	Controls from general practices; no history of previous cancer	592	8.3	6.2, 10.8	*TT* vs. *CC*	1.23	0.81, 1.88		155
							CT vs. *CC*	0.83	0.65, 1.07		
United States	Men enrolled in the Health Professionals Follow-up Study in 1986 who provided a blood sample in 1993–1994; self-reported colorectal cancer, confirmed from medical records and diagnosed in 1986–1994; aged 40–75 years at enrollment in 1986; cohort predominantly White	144	Male controls selected from the same cohort from among those who provided a blood sample in 1993–1994 but who did not report a diagnosis of colorectal cancer	627	13.4	10.8, 16.3	*TT* vs. *CT*/*CC*	0.57	0.30, 1.06	Age, family history, and intake of folate, methionine, and alcohol	149

United States	Male physicians participating in Physicians Health Study trial (exclusion criteria included history of myocardial infarction, stroke or ischemic heart disease, cancer, current renal or liver disease, peptic ulcer, or gout) who provided a blood sample at baseline in 1982 and reported colorectal cancer in 1982–1985, which was confirmed in medical records; mean age, 60 (standard deviation, 9) years	202	Male controls selected from the same cohort, matched to cases on age and smoking status; alive and free of colorectal cancer when matched case was diagnosed; mean age, 57 (standard deviation, 8) years	326	15.0	11.3, 19.4	*TT* vs. *CC*	0.45	0.24, 0.86	Age, smoking status, alcohol intake, multivitamin use, exercise, body mass index, aspirin use	17
							CT vs. *CC*	0.98	0.67, 1.45		
United States: North Carolina	Persons with first invasive colon adenocarcinoma diagnosed in July 1996–June 2000, identified from cancer registry, aged 40–85 years at diagnosis, and had driver's license if under age 65 years; response rate, 66%; 52% male; 44% reported being African-American, 56% as White	552	Controls selected from 1) motor vehicle records (under age 65 years) or 2) lists of Medicare-eligible beneficiaries (aged ≥65 years); frequency matched to cases on ethnic group, age, sex; 38% African American, 62% White	868	6.6§	5.0, 8.4	*TT* vs. *CC*	0.8	0.5, 1.4	Age, ethnic group, sex, sampling fractions	154
							CT vs. *CC*	1.1	0.9, 1.4		
United States: Utah and Minnesota	Participants in KPMCP* and residents of eight counties of Utah and Twin Cities area of Minnesota diagnosed with first primary colon cancer in 1991–1994; aged 30–74 years at diagnosis; 56% male; ethnic group of entire study population 4.2% Black, 4.4% Hispanic, 91.4% White; 75% of cases and controls genotyped	1,467	Controls 1) randomly selected from KPMCP lists, and 2) identified by random digit dialing and lists with driver's license or state identification in Minnesota and Utah (under age 65 years) and 3) randomly selected from Medical Care Financing lists in Utah (aged ≥65 years)	1,821	11.4	9.9, 12.9	*TT* vs. *CC*	0.9	0.7, 1.1	Age, body mass index, long-term vigorous physical activity, energy intake, dietary fiber, usual no. of cigarettes smoked	150
							CT vs. *CC*	1.0	0.9, 1.2		

Table continues

TABLE 2. Continued

Study area	Cases		Comparison group				Comparison	Relative risk	95% CI	Adjustment factors	Reference no.
	Type	No.	Type	No.	% *TT*	95% CI					
United States: Hawaii	Persons with primary adenocarcinoma of the colon or rectum diagnosed in 1994–1998; identified through tumor registry; at least 75% Japanese or Caucasian or any percentage Hawaiian ancestry; 61% male; median age, 66 years; 59% Japanese, 27% Caucasian, 14% Hawaiian	548	Controls selected from 1) participants in an ongoing health survey among a 2% random sample of state households and 2) for those over age 65 years, Health Care Financing Administration rolls; 61% Japanese, 26% Caucasian, 13% Hawaiian	656	15.9¶	13.1, 18.9	*TT* vs. *CC*	0.7	0.5, 1.0	Age, sex, ethnicity, smoking, physical activity, aspirin use, body mass index, schooling, intakes of nonstarch polysaccharides and calcium	54
							CT vs. *CC*	0.8	0.6, 1.1		

* MTHFR, methylenetetrahydrofolate reductase; CI, confidence interval; KPMCP, Kaiser Permanente Medical Care Program.
† DNA source: tumor for cases, blood for controls.
‡ Unmatched odds ratio, computed by Sharp and Little from data in the paper.
§ % *TT*: African Americans = 1.8 (95% CI: 0.7, 3.9); Whites = 9.5 (95% CI: 7.1, 12.3).
¶ % *TT*: Japanese = 19.4 (95% CI: 15.6, 23.6); Caucasian = 14.0 (95% CI: 9.2, 20.2); Hawaiian = 3.4 (95% CI: 0.9, 9.6).

variant (*TT*) subjects compared with homozygotes for the common allele (17, 54, 149–151, 153, 154). Observed relative risks ranged from 0.45 to 0.9, although most did not reach statistical significance. A significant trend of decreasing risk with increasing number of *T* alleles has been reported (54). As has been observed in several meta-analyses of gene-disease associations (156, 157), the strongest effects were found in the two earliest studies (17, 149). Both were nested within cohort studies of predominantly White male populations in the United States. These populations were likely to have relatively high average intakes of total folate as a consequence of comparatively frequent use of vitamin supplements (158).

Although two studies were null overall (98, 155), one found an association with genotype in a subgroup (refer to the information later in this section; Shannon et al. (98)). In the other, although controls were matched to cases on age, sex, and general practice, this matching was not taken into account in the *MTHFR* analysis (155). The distribution by area of residence, which determines general practice, differed between cases and controls; if the prevalence of *MTHFR* variants differed between areas, this lack of adjustment could have affected the results. In addition, the *TT* prevalence among controls was lower than that in other studies from the United Kingdom.

In a study in Mexico, a nonsignificantly increased risk in carriers of the *T* allele was reported (152). This finding was based on small numbers of subjects, few details were provided about subject source populations, and the source of the DNA was tumor for cases and blood for controls.

One study observed that the inverse association with the *TT* genotype was stronger in older (aged 60–84 years) than in younger (aged 40–59 years) subjects, but this finding was not statistically significant (17). The same study reported that the inverse association held for tumors in both the colon and the rectum. In terms of location in the colon, Slattery et al. (150) found that the *TT* genotype was associated with reduced risk in persons with proximal, but not those with distal, tumors. Two studies report results by ethnic group. Le Marchand et al. (54) found that the *TT* genotype was inversely associated with risk for subjects of Japanese origin and Caucasians, but not for Hawaiians. However, only nine Hawaiian subjects had the *TT* genotype. Keku et al. (154) found a modest inverse association among White subjects and African-American subjects.

Shannon et al. (98) stratified their cases into those showing microsatellite instability (MSI+) and those not (MSI–). *TT* genotype was associated with significantly raised risk in the MSI+ group (unadjusted odds ratio (OR) computed by us for *TT* vs. *CC* = 2.6, 95 percent CI: 1.08, 5.82) but not in the MSI– group. The MSI+ tumors were exclusively in the proximal colon and patients tended to be older, both factors that might have been expected to result in a reduced risk in *TT* subjects if the above observations regarding age and tumor location are true. This apparent inconsistency may be due to small numbers, bias, a failure to control for confounders, or chance. Further investigation to unravel the independent and joint influences of MSI, age, and tumor site is needed.

We know of six studies that have investigated *C677T* and adenomatous polyps, three in the United States and one each

in Japan, Norway, and Mexico (76, 152, 159–162; table 3). None found a significant association between genotype and risk, which raises the possibility that the *MTHFR* genotype may be relevant only in the later stages of the adenoma-carcinoma process, for example, in determining those persons with adenomas who will go on to develop carcinomas. It is also possible that the inconsistencies between the results of the studies of adenomas are due to differences between the studies in the subject source populations (i.e., whether they included screen-detected or symptomatic adenomas) and in the control series (e.g., whether it comprised polyp-free subjects).

In two studies of hyperplastic polyps, no association was found between genotype and disease (162, 163; table 4).

A1298C. Four studies, three in the United States (28, 54, 154) and one in Scotland (56), have investigated the role of *A1298C* in cancer (table 5). In all, risk was modestly reduced in *CC* compared with *AA* subjects. Relative risks were in the range of 0.6–0.8 and mostly did not reach statistical significance. Since this finding is consistent with the pattern observed for *C677T*, it raises the possibility that the *A1298C*-cancer relation is actually due to *C677T*. However, Chen et al. (28) reported that the *A1298C* result was not due to confounding by *C677T*. In addition, Le Marchand et al. (54) found that, compared with *677CC/1298AA* persons, those who carried *677T* and *1298C* had the lowest risk. Keku et al. (154) reported that the *A1298C*-cancer association was stronger among White than African-American subjects.

MTR

One cancer study and one of adenomas found a slightly reduced risk for *GG* homozygotes (18, 76; table 5). A third study found no effect overall but observed an inverse association between *GG* and cancer among a subgroup of Hawaiian subjects (54).

MTRR

In the single study that we know of, in Hawaii, *A66G* was not associated with cancer when the three ethnic groups included in the study were analyzed together (54; table 5). However, among White subjects, there was a trend of borderline significance of increasing risk with increasing number of variant alleles (OR for *GG* vs. *AA* = 1.9, 95 percent CI: 1.0, 3.8; p for trend = 0.07).

CBS

Heterozygotes for the *CBS* insertion were twice as frequent among controls as among cancer cases in one study (OR computed by us = 0.50, 95 percent CI: 0.24, 1.07) (98; table 5). Compatible with this finding, the other available study suggested that the variant was associated with reduced cancer risk (54).

TS

In the single study that we are aware of, of the 6-base-pair deletion and cancer in non-Hispanic White subjects in the United States, which was reported in abstract form only, subjects with the deletion had a relative risk of 1.40 (95 percent CI: 0.99, 1.98; $p = 0.058$) compared with those with no deletion allele (164; table 5). In another study of men in the United States, again reported only as an abstract, 2 rpt homozygous persons had a nonsignificantly reduced cancer risk (relative risk for 2 rpt/2 rpt vs. 3 rpt/3 rpt = 0.65, 95 percent CI: 0.38, 1.12) (99). In the single study of adenomas, no significant association was found between either polymorphism and disease, nor did combinations of the two polymorphisms affect risk (102).

Other diseases

Genetic variation in *MTHFR*, *CBS*, *MTR*, *MTRR*, and *TS* has been investigated in other conditions in which folate or homocysteine may be involved. Examples are congenital anomalies such as neural tube defects, Down's syndrome, and orofacial clefts (5, 40, 49, 84, 165, 166); cancers including leukemia and lymphomas, breast, gastric, and esophageal tumors (50, 55, 64, 67, 167); cardiovascular disease (34, 87, 158, 168, 169); and Alzheimer's disease (170).

INTERACTIONS

Gene-environment interactions

MTHFR C677T. The gene-environment interactions explored have concerned features of the "low-methyl" diet and genotype. Four of five studies suggest interactions between folate, methionine, or alcohol and *C677T* in relation to cancer. Chen et al. (149) reported that the inverse association with the *TT* genotype was greatest among persons in the highest tertiles of folate and methionine intake. The results of Ma et al. (17), who examined plasma folate, and Le Marchand et al. (54), who analyzed food and total folate intake, were compatible with this finding. Keku et al. (154), however, did not observe this pattern with regard to total folate intake.

Slattery et al. (150) categorized subjects as consuming low-, intermediate-, and high-methyl diets. The lowest odds ratio was for subjects with the *TT* genotype consuming a high-methyl diet (OR for high-methyl and *TT* vs. low-methyl and *CC* = 0.4, 95 percent CI: 0.1, 0.9), while the odds ratios for subjects consuming a low-methyl diet did not vary by genotype (150). Consistent with this finding, Ma et al. (17) observed an increased risk among the folate deficient (plasma folate <3.0 ng/ml) irrespective of genotype.

Two cancer studies found significant interactions between *C677T* and alcohol (17, 149). High intake abolished the reduced risk associated with the *TT* genotype to the extent that subjects with this *TT* genotype who consumed the largest quantities of alcohol were at the greatest risk of cancer (greater even than for those without the *T* allele who

TABLE 3. Studies of the *MTHFR C677T* genotype and adenomatous polyps, with relative risks and 95% confidence intervals**

Study area	Cases		Comparison group				Comparison	Relative risk	95% CI*	Adjustment factors	Reference no.
	Type	No.	Type	No.	% *TT*	95% CI					
United States	Women enrolled in the Nurses' Health Study in 1976 who provided a blood sample in 1989–1990; first incident proximal or distal colorectal adenoma diagnosed during time from blood specimen to June 1994; approximately 95% of the cohort is White	257	1) Cohort members in whom colorectal adenoma had not been diagnosed and who were born in the same year as the matched case and had had a sigmoidoscopy since the blood sample was taken (n = 257); plus 2) female cohort members who had served as controls for a breast cancer study, of whom 71% had not had a sigmoidoscopy and 29% had had a sigmoidoscopy and did not have an adenoma	713	9.3	7.2, 11.6	*TT* vs. *CT*/*CC*	1.35	0.84, 21.7	Age, family history, smoking status, body mass index, and intakes of folate, methionine, alcohol, fiber, and saturated fat	76
United States: California	Subjects undergoing screening sigmoidoscopy at two medical centers during 1991–1993; aged 50–74 years; no evidence of prior bowel disease and no previous bowel surgery										160
	Diagnosed for the first time with one or more histologically confirmed adenomas; 65% male; 55% White, 17% Black, 17% Hispanic, 11% Asian; mean age, 67 years	471	Without any adenoma at sigmoidoscopy and no history of adenomas; matched to cases on sex, sex, date of sigmoidoscopy, and clinic	510	9.6	7.2, 12.5	*TT* vs. *CC*	1.11	0.71, 1.71	Age, race, sex, clinic, date of sigmoidoscopy	
							CT vs. *CC*	0.85	0.65, 1.13		
United States: Minneapolis, Minnesota	Subjects recruited from private gastroenterology practice undertaking colonoscopies in 10 hospitals; underwent colonoscopy in 1991–1994; English speaking; no known genetic syndromes predisposing to colorectal cancer; no history of cancer or inflammatory bowel disease; aged 30–74 years; participation rate, 68%										159
	Subjects with first diagnosis of colon or rectal adenomas; 62% male; mean age, 58.1 (standard deviation, 9.7) years; 98% White	527	Free of all polyps at colonoscopy; 38% male; mean age, 52.8 (standard deviation, 10.9) years; 97% White	645	11.0	8.7, 13.7	*TT* vs. *CC*	0.8	0.5, 1.3	Age, sex, body mass index, use of hormone replacement therapy, and percentage of calories from fat, dietary fiber, folate, vitamin B_{12}, vitamin B_6, methionine, alcohol	
							CT vs. *CC*	0.9	0.7, 1.2		
Japan	Male military officials undergoing preretirement health examination at two hospitals; had a partial or total colonoscopy and provided a blood sample; aged 47–55 years; no prior history of colectomy, polypectomy, malignant neoplasia										161
	Histologically confirmed colorectal adenoma without in situ or invasive carcinoma	205	Normal total colonoscopy	220	11.8	7.9, 16.8	*TT* vs. *CC*	0.87	0.56, 1.34	Hospital, employment, military rank, smoking, alcohol intake	
							CT vs. *CC*	1.17	0.61, 2.23		

Norway	Participants in Telemark I study; born in 1924–1933; selected from population register in 1983 and randomly assigned to endoscopy or control group; 799 participated; in 1996, offered colonoscopy and removal of polyps; results available for 443 participants (229 male, 214 female; median age, 67 years)												162
	Subjects with "high-risk" colorectal adenomas (≥10 mm or severe dysplasia or villous components)	47	Without polyps (n = 116) or with hyperplastic polyps or "low-risk" adenomas (n = 278)	394	7.1	4.8, 10.1	*TT* vs. *CC*	2.41	0.82, 7.06	Age, sex, red blood cell folate, use of nonsteroidal antiinflammatory drugs, flexible sigmoidoscopy in 1983, body mass index, current smoking			
							CT vs. *CC*	1.51	0.76, 2.99				
Mexico	Patients with colorectal adenomas	32	"Asymptomatic" subjects; source not stated	110	21.8	14.5, 30.7	*TT* vs. *CC*	1.65†	0.41, 6.73				152
							CT vs. *CC*	0.98†	0.28, 3.67				

* MTHFR, methylenetetrahydrofolate reductase; CI, confidence interval.
† Unmatched odds ratio, computed by Sharp and Little from data in the paper.

were in the highest alcohol group). Keku et al. (154) found no interaction with alcohol but did not consider quantity, only whether subjects had "ever" or "never" consumed alcohol.

High blood riboflavin levels may improve MTHFR activity in *TT* persons because the cofactor for MTHFR is a metabolite of riboflavin (171). Le Marchand et al. (54) observed the lowest relative risk for cancer among *TT* persons with the highest riboflavin intake. Genotype-folate-riboflavin combinations were not considered.

Little is published on gene-diet interactions and adenomas. In the two known studies, the stratum of highest risk comprised *TT* persons with the lowest red cell or plasma folate levels (160) or the lowest intakes of folate, methionine, vitamin B_6, and vitamin B_{12} (159), but the gene-nutrient interactions were not statistically significant. With regard to alcohol and genotype, the pattern observed is similar to that for cancer (159, 160).

MTHFR A1298C. Keku et al. (154) observed a significant interaction (p = 0.03) between total folate intake and *A1298C* genotype among White but not African-American subjects; fewer African-American subjects were involved in the study. Unlike the pattern for *C677T*, White *1298CC* subjects who consumed less than 400 ng of folate per day had a greater reduced cancer risk than those whose folate intake was higher. No interactions were observed between *A1298C* and "ever" or "never" consuming alcohol.

Two further studies of *A1298C* reported no significant interactions with blood levels or intake of folate or related nutrients and colorectal neoplasia (28, 54). The results were not shown.

MTR. For cancer, Ma et al. (18) reported a significant interaction between *MTR* and alcohol intake (table 5); persons with the *GG* genotype consuming more than one drink a day had an increased disease risk (OR for *GG* and ≥1 drink/day vs. *AA* and <1 drink/day = 2.64, 95 percent CI: 0.65, 10.82), while those consuming less than one drink a day had a reduced risk (OR = 0.27, 95 percent CI: 0.09, 0.81; p for interaction = 0.04). There was also a nonsignificant 50 percent risk reduction among *GG* subjects whose plasma folate levels were in the upper two tertiles compared with those with the same folate level and the *AA*/*AG* genotype; persons with the *GG* genotype in the lowest plasma folate tertile did not have a reduced risk (p for interaction = 0.22).

MTRR and CBS. Le Marchand et al. (54) reported no significant interactions between *MTRR* or *CBS* and dietary folate, vitamin B_{12}, vitamin B_6, riboflavin, or methionine. Results were not shown.

TS. For adenomas, Ulrich et al. (102) found a statistically significant interaction between the tandem repeat polymorphism and folate intake. Among 3 rpt/3 rpt persons, higher folate intake (>440 ng/day) was associated with a 50 percent reduced risk compared with lower folate intake. However, among 2 rpt/2 rpt persons, higher folate intake was associated with a 50 percent increased risk (p for interaction = 0.03). A similar pattern was observed for vitamin B_{12} intake (p for interaction = 0.08). No interactions were found with intakes of vitamin B_6, methionine, or alcohol, nor were there interactions between the 3′ untranslated region polymorphism and dietary variables.

TABLE 4. Studies of the *MTHFR *C677T* genotype and hyperplastic polyps, with relative risks and 95% confidence intervals**

Study area	Cases		Comparison group				Comparison	Relative risk	95% CI*	Adjustment factors	Reference no.
	Type	No.	Type	No.	% *TT*	95% CI					
Norway	Participants in Telemark I study; born 1924–1933; selected from population register in 1983 and randomly assigned to endoscopy or control group; 799 participated; in 1996, offered colonoscopy and removal of polyps; results available for 443 participants (229 male, 214 female; median age, 67 years)										162
	With "high-risk" hyperplastic polyps ($n \geq 3$)	91	Without polyps ($n = 116$) or with adenomas or "low-risk" hyperplastic polyps ($n = 233$)	349	7.1	4.8, 10.1	*TT*/*CT* vs. *CC*	1.43†	0.87, 2.33		
United States: Minneapolis, Minnesota	Subjects recruited from private gastroenterology practice undertaking colonoscopies in 10 hospitals; underwent colonoscopy in 1991–1994; English speaking; without known genetic syndromes predisposing to colorectal cancer; no history of cancer or inflammatory bowel disease; aged 30–74 years										163
	Diagnosis of colon or rectal hyperplastic polyps; 97% White; 57% male; mean age, 53.7 years	200	Free of all polyps at colonoscopy; 97% White; 38% male; mean age, 52.8 (standard deviation, 10.9) years	645	11.0	8.7, 13.7	*TT* vs. *CC*	0.9	0.5, 1.6	Age, sex, body mass index, use of hormone replacement therapy, smoking, percentage of calories from fat, dietary fiber, folate, vitamin B_{12}, vitamin B_6, methionine, alcohol	
							CT vs. *CC*	0.8	0.6, 1.2		

* MTHFR, methylenetetrahydrofolate reductase; CI, confidence interval.
† Unmatched odds ratio, computed by Sharp and Little from data in the paper.

Gene-gene interactions

Metabolism of any exposure is likely to depend on the balance between the relative activities of all of the enzymes active within the metabolic pathway (172). So far, we know of two studies that have considered joint effects of folate-pathway genes (54, 102; table 5).

For cancer, Le Marchand et al. (54) observed that the *MTHFR T* allele had the greatest effect among subjects with the *MTR G* allele (OR for *CT*/*TT* and *AG*/*GG* vs. *CC* and *AA* = 0.7, 95 percent CI: 0.5, 1.0; p for interaction = 0.05). Considering *MTHFR C677T* and *CBS*, they reported that the group with both variants appeared to be at reduced risk; however, this result was based on small numbers, and the interaction was not significant. Meanwhile, *MTRR* did not interact with *MTHFR C677T*.

For adenomas, Ulrich et al. (102) investigated interactions between *C677T*, *TS* tandem repeat, and folate intake. The association of higher folate intake with reduced risk among 3 rpt/3 rpt subjects was not modified by *MTHFR*. The increased risk associated with lower folate intake in *TT* subjects appeared limited to 3 rpt homozygotes. These findings were not statistically significant.

Comments on studies of gene-disease associations and interactions

Some of the heterogeneity in the findings with regard to the genotype main effects is likely to be due to differences between the populations studied in average levels of intake of folate, alcohol, and related dietary factors. If there truly are interactions between genotype and folate, for example, they may be seen only in populations with high or low folate levels (depending on the direction of the interaction). Such an effect has recently been observed for *MTHFR C677T* and coronary heart disease (158).

Methodological factors are also important. Five cancer studies (17, 56, 149, 151, 152) and four adenoma studies (76, 152, 161, 162) each included fewer than 300 cases and thus had limited statistical power, particularly for subgroup and interaction analyses. The nonprospective studies are most susceptible to bias. Some were not population based. In some, it is not clear whether the controls came from the population that gave rise to the cases. In others, the case series were limited to subjects still alive to provide a DNA sample (prevalent cases), which would have resulted in bias if any of the genotypes were associated with survival (currently not known). Few studies provided information on

participation rates, making it difficult to assess bias and generalizability. It is likely that a proportion of the controls in the cancer studies may have been harboring undiagnosed polyps. Depending on the relations between each polymorphism and adenomas, this may have introduced random error or bias. The presence of undetected polyps among controls would not be important if the genotype was etiologically relevant only *after* an adenoma had developed, as seems likely for *MTHFR C677T*. For the other genotypes, it is not clear at what stage in the adenoma-carcinoma sequence they may be relevant. Finally, the possibility cannot be discounted that the findings do not reflect an association between the specified polymorphisms and colorectal neoplasia but rather are a consequence of linkage disequilibrium.

LABORATORY TESTS

MTHFR C677T and *A1298C* are detected by means of DNA amplification using polymerase chain reaction followed by restriction fragment length polymorphism analysis; *HinfI* for *C677T* and *MboII* (12) for *A1298C* (10, 11) are used. The *MTR* and *MTRR* polymorphisms and the 3′ untranslated region variant in *TS* are also detected by restriction fragment length polymorphism, with digestion with *MaeII* for *MTR*, with *NdeI* or *AflIII* for *MTRR*, and with *DraI* for *TS* (4, 5, 48, 54). The *TS* tandem repeat and *CBS* insertion are detected by DNA amplification and visualization on agarose gels (46, 97).

Most studies did not report the success rate in extracting DNA from samples, the proportion of eligible subjects for whom genotyping failed, or the degree of genotyping reproducibility, all of which are important indicators of the analytical validity of genotyping (173).

Laboratories are increasingly using high-throughput genotyping methods, an area of considerable development and innovation. Although quality control and analytical validity in this context are important (173), published data are currently lacking.

POPULATION TESTING

Companies in the United States and the United Kingdom are offering consumer tests for genotypic or phenotypic markers of polymorphisms influencing nutrient metabolism, including *MTHFR* (174, 175). However, the scientific evidence currently is not strong enough to advocate population testing for any polymorphisms reviewed here.

Testing for these polymorphisms might be valuable in cancer patients. 5-Fluorouracil, commonly used in colorectal cancer chemotherapy, is a thymidylate synthase inhibitor and can cause severe folate depletion. Knowledge of patient genotype could be used to tailor chemotherapy regimes to 1) minimize toxicity and side effects, thus improving quality of life, and/or 2) increase the effectiveness of treatment and ultimately lengthen survival. So far, evidence in this area is limited to the *TS* tandem repeat and *MTHFR C677T*. Among 51 stage III colon cancer patients treated with 5-fluorouracil and leucovorin (folinic acid), presence of the *MTHFR T* allele had little effect on probability of death or length of survival in those who had died, except in 12 patients with rectosigmoid colon cancer (176). In a study of 365 nonadjuvant-treated patients, the *TT* genotype was associated with improved survival, but this result did not persist after adjustment for disease stage (98).

For *TS*, some (177–179) but not all (180, 181) studies of colorectal cancer patients concluded that higher *TS* tumor expression levels were related to shorter survival. Consistent with this finding, one genotype study suggested that carrying the 3 rpt allele increased risk of death (179). Four studies of genotype and response to 5-fluorouracil (182–185) suggested that 2 rpt/2 rpt patients may be more responsive to therapy but subject to greater toxicity (186). Most of the studies (of genotype or phenotype) have been small, included selected patient groups, and made limited adjustment for potentially important factors.

CONCLUSIONS AND RESEARCH PRIORITIES

The observed association of the *MTHFR* homozygous variant genotypes with reduced carcinoma risk was the opposite of what might have been expected a priori. This finding has led investigators to reconsider the folate metabolism pathway, putting a greater emphasis on the functions of folate and MTHFR in DNA synthesis. The evidence is compatible with interactions between *MTHFR* genotype and folate, alcohol, and/or related nutrients in relation to colorectal cancer. Evidence on polymorphisms other than *MTHFR C677T* is extremely limited. The associations observed between *MTR*, *CBS*, *MTRR*, and *TS* genotypes and colorectal neoplasia are tentative at best and require replication. The few studies of combinations of polymorphisms suggest the possibility of gene-gene interactions; again, further investigation is needed to confirm initial findings. Altogether, the evidence suggests that the roles of folate-metabolizing genes, folate, and related dietary factors in colorectal neoplasia are complex. Methodologies are currently lacking for specification of hypotheses, clarification of functional effects, and statistical analysis relating to such complex gene-environment pathways. This area of research must be a priority if advancements in understanding of disease etiology are to be achieved. Table 6 lists other areas for further research.

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TABLE 5. Summary of studies of the *MTHFR *A1928C* polymorphism, other folate pathway genes, and colorectal neoplasia**

Gene	Polymorphism	Study area, study design, cases†	Gene-disease associations			Gene-environment interaction	Gene-gene interactions	Reference no.
			Comparison	Relative risk	95% CI*			
MTHFR	*A1298C*	United States, case-control, carcinoma	*CC* vs. *AA*	0.8	0.5, 1.4	No interactions with total or dietary folate, vitamin B_6, vitamin B_{12}, riboflavin, methionine, ethanol‡		54
		United States, nested case-control, carcinoma	*CC* vs. *AA*	0.73	0.37, 1.43	Risk associated with *CC* not modified by plasma folate status‡		17, 28
		United States, case-control, carcinoma	*CC* vs. *AA*	0.6	0.4, 0.9	Significant interaction between *A1298C* and total folate intake for Whites only; among African Americans, combined *C677T* and *A1298C* genotype and total folate produced interaction of borderline significance; no significant interactions of *A1298C* and alcohol intake for either ethnic group		154
		Scotland, case-control, carcinoma	*CC* vs. *AA*	0.67	0.39, 1.13	—§		56
*MTR**	*A2756G*	United States, nested case-control, adenoma	*GG* vs. *AA*	0.66	0.26, 1.70	—§	No significant interaction with *MTHFR C677T*‡	76
		United States, case-control, carcinoma	*GG* vs. *AA*	1.1	0.6, 2.2	No interactions with total or dietary folate, vitamin B_6, vitamin B_{12}, riboflavin, methionine, ethanol‡	Significant interaction with *MTHFR C677T*	54
		United States, nested case-control, carcinoma	*GG* vs. *AA*	0.59	0.27, 1.27	Significant interaction with alcohol; suggestion of possible joint effect with plasma folate, but not significant; no interaction with homocysteine; no significant interaction with vitamin B_{12}‡	—§	18

Table continues

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TABLE 5. Continued

Gene	Polymorphism	Study area, study design, cases†	Gene-disease associations			Gene-environment interaction	Gene-gene interactions	Reference no.
			Comparison	Relative risk	95% CI*			
*MTRR**	*A66G*	United States, case-control, carcinoma	*GG* vs. *AA*	1.4	0.9, 2.0	No interactions with total or dietary folate, vitamin B_6, vitamin B_{12}, riboflavin, methionine, ethanol‡	No interaction with *MTHFR C677T*	54
*CBS**	68 bp* insertion	United States, case-control, carcinoma	Weak inverse association with presence of insertion¶			No interactions with total or dietary folate, vitamin B_6, vitamin B_{12}, riboflavin, methionine, ethanol‡	Weak suggestion of possible interaction with *MTHFR C677T*	54
		Australia, case-control, carcinoma#	Frequency of heterozygotes in controls (10%) vs. cases (5%)			—§		98
*TS**	28 bp tandem repeat	United States, case-control, adenoma	2 rpt*/2 rpt vs.	0.9	0.6, 1.3	Significant interaction with total folate intake; borderline significant interaction with total vitamin B_{12} intake	Suggestion of joint effect with *MTHFR C677T*	102
			3 rpt/3 rpt			No interactions with vitamin B_6, methionine, or alcohol‡		
	28 bp tandem repeat	United States, case-control, carcinoma	2 rpt/2 rpt vs. 3 rpt/3 rpt	0.65	0.38, 1.12			99
	6 bp deletion in 3′ untranslated region	United States, case-control, carcinoma	With deletion vs. no deletion	1.40	0.99, 1.98			164
	6 bp deletion in 3′ untranslated region	United States, case-control, adenoma	Homozygous no deletion vs. 6 bp/6 bp	1.13	0.73, 1.74	No consistent patterns with dietary folate or vitamin B_{12}‡	No consistent patterns with *MTHFR C677T*‡	102

* MTHFR, methylenetetrahydrofolate reductase; CI, confidence interval; MTR, methionine synthase; MTRR, methionine synthase reductase; CBS, cystathionine β-synthase; bp; base pair; TS, thymidylate synthase; rpt, repeat.

† Refer to tables 2 and 3 for further details of the study populations, etc.

‡ Data not shown in the paper.

§ None mentioned in the paper as having been investigated.

¶ Unadjusted odds ratio for heterozygotes or homozygous insertion vs. homozygous no insertion = 0.88 (95% CI: 0.50, 1.55).

This analysis included only 155 of the original control series.

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TABLE 6. Research priorities

1. Further documentation of genotype frequencies: large, population-based studies of the polymorphisms reported in this paper and the additional, but less well studied, polymorphisms in these genes (e.g., *G1793A* in *MTHFR*), including prevalences of combinations of polymorphisms and prevalence in different age groups; particularly needed in non-White populations and less-investigated ethnic groups in the United States and Europe
2. Clarification of functional effects of the polymorphisms: including exploration of 1) consequences of carrying combinations of polymorphisms in both in vivo and in vitro systems and 2) in vivo functional effects of particular genotypes in persons with different levels of intake of folate and related dietary factors
3. Further investigation of hypothesized mechanisms: examination of whether the polymorphisms are associated, in humans, with genomic DNA methylation, uracil incorporation, or DNA strand breaks, including exploration of whether relations differ according to levels of folate and related dietary factors
4. Studies of gene-disease associations and gene-environment and gene-gene interactions: further large population-based studies of polymorphisms and cancer and adenomas, incorporating collection of high-quality dietary data and, ideally, blood biomarkers; these studies should be large enough to have sufficient power to investigate gene-environment and gene-gene interactions and to undertake subgroup analysis by age and ethnic group, of colon and rectal tumors, proximal and distal tumors, and tumors with microsatellite instability or loss of heterozygosity
5. Pooled analyses of studies of gene-disease associations and gene-environment and gene-gene interactions to facilitate subgroup analyses and investigation of interactions
6. Investigation of the role of other folate pathway genes, and interactions with alcohol-metabolizing genes, in the etiology of colorectal neoplasia
7. Investigation of genotype in adenomatous polyps: including 1) association with risk of recurrence and 2) association with particular pathologic features and 3) incorporation of genotyping in randomized controlled trials of folate supplementation in prevention of colorectal neoplasia
8. Further investigation of genotype and quality of life and the effectiveness of treatment in patients with colorectal cancers: large studies of representative groups of patients; analysis should include adjustment for known prognostic factors
9. Development of methodology for specifying hypotheses and statistical analysis in the context of interactions between multiple genes and multiple environmental factors

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APPENDIX. Internet sites pertaining to folate metabolism and colorectal neoplasia

Data on cancer incidence, survival, and mortality

International Agency for Research on Cancer (IARC)—Cancer Mondial:
http://www-dep.iarc.fr/dataava/infodata.htm
Surveillance, Epidemiology, and End Results (SEER) Program:
http://www.seer.cancer.gov/publicdata/
National Program of Cancer Registries (NPCR):
http://www.cdc.gov/cancer/npcr

Information on cancer

Cancer Research UK:
http://www.cancerresearchuk.org/
National Cancer Institute—cancer.gov:
http://www.nci.nih.gov/
American Cancer Society:
http://www.cancer.org/docroot/home/index.asp

Genetics information

Human Genome Epidemiology Network (HuGENet):
http://www.cdc.gov/genomics/hugenet/default.htm
Public Health Genetics Unit:
http://www.medschl.cam.ac.uk/phgu/
Online Mendelian Inheritance in Man (OMIM):
http://www3.ncbi.nlm.nih.gov/Omim/searchomim.html
GenAtlas:
http://www.dsi.univ-paris5.fr/genatlas
GeneCards:
http://www.cgal.icnet.uk/genecards
National Center for Biotechnology Information:
http://www.ncbi.nlm.nih.gov/
UK Human Genome Mapping Project (includes links to other sites via The Genome Web):
http://www.hgmp.mrc.ac.uk/